42. (Amended) A method according to Claim 29, wherein said contacting the resulting combination step further comprises binding VEGF present in the sample with expressed VEGF receptor.

43. (Amended) A method according to Claim 42, wherein said contacting the resulting combination step further comprises activating MAPK with the expressed VEGF receptor.

49. (Amended) A method according to Claim 29, wherein the sample comprises cells, tissue, tissue extracts, or combinations thereof.

REMARKS:

Priority

The Applicants appreciate the Examiner's comments regarding priority under 35U.S.C. §119(e). In accordance with 35U.S.C. §119(e)(1), the applicants have submitted an amendment containing a specific reference to the earlier filed applications in the section above entitled "In the Specification". The Applicants note that a petition and surcharge are not required to correct this claim, because the Patent Office recognized the Applicants' priority claim, which was included in the oath or declaration, by its inclusion on the filing receipt.

Specification

The second paragraph on page 4 and the paragraph that begins on page 4, line 26 have been amended to correct for obvious typographical errors which were pointed out by the Examiner. The third full paragraph on page 8 has also been amended to correct for an obvious typographical error.

Claims Amendments

Claim 1 and Claim 29 have been amended to be more clear by deleting a repeated step in Claim 1 and Claim 29. Claim 1 has also been amended to make it easier to read by indenting and assigning letters to the steps of the method. Claim 29 has been amended to make it easier to read by indenting and assigning letters to the steps of the method and to clarify what is claimed. Claims 1 and 29 have also been amended to clarify that the stable cell line of the invention is a

HeLa cell line. Claims 21 and 49 have been amended by replacing "and" with "or" as suggested by the Examiner to clearly indicate that the cells, tissue, and tissue extracts can be used independently as well as in combination. Claims 1, 7, 9, 14, 15, 22-25, 29, 35, 37, 42 and 43 have been amended for various reasons to comply with 35 USC §112. Claims 9 and 37 have been amended to correct for an obvious typographical error. Claims 30 and 31 have been amended to depend from Claim 29 instead of cancelled Claim 26.

Cancelled Claims

Claims 26-28 have been cancelled to expedite prosecution of the remaining claims. Claims 13 and 41 have been cancelled because they are redundant with amended Claims 1 and 29.

Claims 1-25, 29-40, and 42-50 are currently pending. No new matter has been introduced. Examiner is respectfully requested to enter the amendments and reconsider the application.

Claim Rejections under 35 USC §112, first paragraph

The Examiner has rejected Claims 19 and 47 under 35 USC §112, first paragraph because the claims recite "biological fluids." The Examiner states that "[t]hese examples are widely divergent and do not provide sufficient description to allow one of ordinary skill in the art to [sic] ascertain the identifying characteristics of a biological fluid." However, the Examiner does not cite to any support for the statement that the examples of plasma and cell culture media are "widely divergent." Applicants respectfully submit that given the context of the application, the specification would reasonably convey to one of skill in the art that the inventors had the claimed invention in their possession at the time the application was filed. In other words that plasma and cell culture media are representative of the genus "biological fluids" in the context of the specification as filed.

Claim Rejections under 35 USC §112, second paragraph

The Examiner has rejected claims 1-25, 29 and 35-49 under 35 USC §112, second paragraph because Claims 1 and 29 included the language "a chimeric transactivatable vector."

Claims 7 and 35 also contain this language. Applicants have amended claims 1, 7, 29 and 35 to amend this language to "chimeric transactivator vector" as suggested by the Examiner. Thus, this rejection is no longer applicable.

The Examiner has rejected Claims 1, 7, 14, 24, 25 and 35 under USC §112, second paragraph because they "recite limitations without a preceding definite or indefinite article." Claim 1 has been amended to make it easier to read by indenting and assigning letters to the steps of the method. Claims 7, 24, 25, and 35 have been amended by the insertion of the definite article "the" as suggested by the Examiner. As amended Claims 1, 7, 14, 24, 25, and 35 are not indefinite; and thus, the rejection of these claims on this basis is no longer applicable.

The Examiner has rejected Claims 22 and 23 because "it is not possible to express activity in units of concentration." The Examiner states that he has assumed that "the applicants intended for the claims to be drawn to a method wherein VEGF activity is detectable at a VEGF concentration within the indicated ranges." The applicants thank the Examiner for making this assumption. Claims 22 and 23 have been amended to more clearly reflect what is claimed. As amended Claims 22 and 23 are not indefinite; and thus, the rejection of these claim on this basis is no longer applicable.

The Examiner has rejected Claim 29 because it "is not clear how steps (a) and (b) are related to steps (c) or (d). . .. " Claim 29 has been amended to make it easier to read by indenting and assigning letters the portion of the claim that describes the stable cell line. Claim 29 has also been amended to clarify that there is a cell that expresses VEGF and a stable cell line that is used to assay VEGF. Support for this amendment is found in Example 5. The Examiner's assumption as to the intended meaning of this claim is basically correct. The Examiner has assumed that "the VEGF produced by the cell of step (a) is combined with the compound of step (b) and the cell line of step (c) is contacted with the resultant combination." As amended, claim 29 is not indefinite and thus the rejection of this claim on this basis is no longer applicable.

The Examiner has rejected Claims 9 and 37 for reciting "the limitation 'promoting element' " Claims 9 and 37 have been amended per the Examiner's suggestion to read "promoter

element." As amended, claims 9 and 37 are not indefinite; and thus, the rejection of these claims on this basis is no longer applicable.

The Examiner has rejected Claims 15 and 43 for reciting "the limitation 'said including contacting step' in line 1." Claims 15, which depends from Claim 1, has been amended to replace the language "said including contacting step" with "said contacting the sample step"; and 43 has been amended to replace the language "said including contacting step" with "said contacting the resulting combination step." This is slightly different from the language suggested by the Examiner of "said contacting step" in order to differentiate between the two contacting steps in Claim 29 upon which Claim 43 depends. As amended, Claims 15 and 43 have antecedent basis; and thus, the rejection of these claims on this basis is no longer applicable.

The Examiner has rejected Claims 16 and 44 as having insufficient antecedent basis for the language "the limitation 'transactivator'." As suggested by the Examiner, Claims 1 and 29, upon which Claims 16 and 44 depend, respectively, have been amended to include the term "transactivator." Therefore, Claim 16 and 44 have proper antecedent basis; and thus, the rejection of these claims on this basis is no longer applicable.

The Examiner has rejected Claim 42 as having unclear antecedent basis for the language "said contacting step." Claim 42 has been amended to read "said contacting the resulting combination step" to clarify which contacting step is meant. Therefore, Claim 42 has proper antecedent basis; and thus, the rejection of this claim on this basis is no longer applicable.

For all the reasons stated above, Applicants respectfully request the withdrawal of all the claim rejections under 35 USC §112, 1st and 2nd paragraphs.

Claim Rejection under 35 USC §102

The Examiner has rejected Claim 26 under 35 USC §102(a) as being anticipated by Murata [sic], et al. (2000) *J. Ocular Pharmacol and Ther* 16:383. The Examiner has also rejected Claim 26 under 35 USC §102(b) as being anticipated by either of Wen, et al. (1999)

Biochem Biophys Res Commun 258:713-721 or Ullrich, et al. 1994; WO 94 11499). Applicants have cancelled this claim; and therefore, these rejections are not applicable.

Claim Rejection under 35 USC §103

The Examiner has rejected Claims 1 and 29 under "as being unpatentable over Ullrich in view of Hexdall (1999) *Strategies* volume 1, issue 2 and in further view of Shibuya (1999) *International Congress Series* 1175:25-33." The Examiner has also rejected Claims 2-20, 24, and 30-48 because the further limitations of these claims over Claims 1 and 29 are purportedly taught by Hexdall (1999). Claims 15 and 43 are rejected by the Examiner based on Shibuya. Claim 50 is rejected over Ullrich and Hexdall. Finally, Claims 22 and 23 are rejected over "Ullrich in view of Hexdall as applied in Claim 1 above, and further in view of Wen" ((1999) Biochem and Biophys Res Comm, Vol. 258, pp. 713-721).

Applicants thank the Examiner for such a detailed and clear explanation of the rejections and how and to which claims they apply. However, Applicants respectfully submit that the Examiner has not met his burden for a *prima facie* case of obviousness, namely the requirement that there be a reasonable expectation of success.

Applicants respectfully assert that the Examiner is using hindsight construction to come to a conclusion of obviousness. The Examiner has picked and chosen elements from three different references, Ullrich, Hexdall, and Shibuya to come up with the elements of Applicants' invention, as claimed in amended Claims 1 and 29. The Examiner then adds a fourth reference, Murata, to come to the conclusion that "one would have a reasonable expectation of success." The Examiner also cites to a fifth reference, Wen, in his rejection of Claims 22 and 23. The Examiner is using the disclosure of Applicants' specification to direct the construction of Applicants' invention from multiple references. Such hindsight construction of the invention is not proper and is not proof of obviousness.

The references individually or in any combination do not provide a reasonable expectation of success. While it may have been obvious to try the combination of Applicants'

invention this does not equate with obviousness. Applicants respectfully assert that there would not have been a reasonable expectation of success found in the references that HeLa cells transfected with the three constructs required by the claims of the present invention, would have yielded a cell line capable of quantitative measurement of biologically active VEGF. The Examiner cites Hexdall as disclosing the limitation that the cell line be a HeLa cell line. While Hexdall discloses HeLa cells for use in a reporter assay, it does not describe a cell line which expresses a VEGF receptor as in the stable HeLa cell line of the present invention. Ullrich discloses the use of HeLa cells among a group of at least seven other specifically named cells lines (p 20, lines 1-2). Yet, Ullrich only describes the use of one of these, COS-1 cells, in detail and then only for transient transfection. In addition, as noted by the Examiner, Ullrich does not teach a cell line comprising an expressible reporter element and a DNA binding site disposed adjacent thereto, a chimeric transactivator vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain that binds to the DNA binding site in addition to an expression vector encoding a VEGF receptor. Sibuya discloses NIH3T3 cells transfected with the VEGF receptor FLK-1 and Murata uses cells that endogenously express FLK-1. One of skill in the art may have chosen to try HeLa cells to generate the stable cell line of the present invention, but the references provide no indication as to which of the many available cell lines would work. Given the nature of biological systems, one of skill in the art would not have had a reasonable expectation that HeLa cells, which do not endogenously express VEGF receptor, would provide the necessary signaling components to allow a heterologous VEGF receptor to be expressed and to activate MAP kinase to lead to the phosphorylation of a phosphorylatable protein-DNA binding domain chimera. Applicants assert that obvious to try does not equate with obviousness where there was not a reasonable expectation of success at the time of Applicants' invention. Therefore, the rejections of Claims 1-20, 22-24, 29-48, and 50 under 35 USC §103 are improper and should be withdrawn.

Applicants respectfully request reconsideration and withdrawal of all of the rejections in light of the amendments and remarks made herein and allowance of all the pending claims.

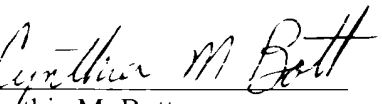
Attached hereto is a marked-up version of the changes made to the claims by the current amendment entitled "Versions with markings to show changes made."

Finally, the undersigned notes that **subsequent correspondence** should be addressed to:

Cynthia M. Bott
Warner-Lambert Company
2800 Plymouth Road
Ann Arbor, MI 48105
(734-622-4182).

Respectfully submitted,

Date: December 4, 2002



Cynthia M. Bott
Registration No. 46,568
Warner-Lambert Company
2800 Plymouth Road
Ann Arbor, MI 48105
Tel. (734) 622-4476
Fax (734) 622-1553

VERSIONS WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

The second paragraph on page 4 has been amended as follows.

The ~~assay~~ methods of the present invention ~~utilizes~~ use a ~~stable~~ VEGF responsive HeLa cell line which is ~~comprises HeLa cells which have been~~ stably transfected with 1) a reporter vector having an expressible reporter element and a DNA binding element disposed adjacent thereto. Preferably, the reporter vector includes a gene encoding a detectable gene product which is disposed downstream of a basic promoter element, preferably a TATA box, which is joined to the binding element which is preferably a GAL4 binding element[.] 2) ~~The stable cell line is also transfected with a vector encoding~~ a CMV promoter-driven vector encoding a fusion protein comosed of the yeast GAL4 binding domain and the transactivation domain of the transcription factor ELK-1 ~~transcription factor (which is tied to the MAP kinase pathway)~~ and ~~is fused to a yeast GAL4 DNA binding domain and a yeast GAL4 binding element-driven luciferase reporter construct.~~ and 3) ~~A third~~ a vector encoding ~~a gene capable of expressing mouse~~ FLK-1 VEGF receptor. ~~is also transfected into the cells and the stable~~ The cell lines ~~generated therefrom~~ of the present invention can be ~~utilized~~ used to demonstrate upregulation ~~for~~ of the detectable gene product (e.g. luciferase ~~expression~~) in the presence of VEGF. That is, utilizing established signal transduction pathways, VEGF bioactivity can be assayed.

The paragraph that begins on page 4, line 26 has been amended as follows:

In general, utilizing known signal transduction relationships and/or pathways, a sample to be assayed for VEGF bioactivity is placed in a container containing the stable cell line as described above. If VEGF is present in the sample, VEGF activates FLK-1 expressed by the stable cell line. Activated FLK-1, which is a known VEGF receptor, then activates MAP kinase (Kroll and Waltenberger, *J. Biol. Chem.*, 1997:272:32521-32527; Doanes et. al., *Biochem. Biophys. Res. Comm.*, 1999;255:545-548). The activated MAP kinase phosphorylates the fusion trans-activation protein (GAL4 DNA binding domain [dbd] fused with ELK-1). The phosphorylated fusion protein binds to the GAL4 DNA binding site of the ~~reported~~ reporter vector activating luciferase expression. Luciferase expression can be detected utilizing techniques well-known in the art. The presence or expression of luciferase indicates VEGF activity in the sample

Please replace the third full paragraph on page 8 with the following:

Figure 10 shows the effects of $AdVEGF_{121}$ obtained from using a media from $AdVEGF_{121}$ transfected rat 2 cells. The addition of $AdVEGF_{121}$ to the VEGF-receptor cell line affected luciferase expression in a dose ~~close~~ response manner both from the $AdVEGF_{121}$ itself and from the media from $AdVEGF_{121}$ transfected rat 2 cells.

The section on page 8 which begin with "Example 6"has been amended as follows:

EXAMPLE 6

~~Luciserase~~ Luciferase Production in VEGF-Receptor Cell Line at 24 and 48 Hours After the Addition of VEGF

Cells were prepared as described above. $VEGF_{121}$ was added to cells (50 K/well) 24 hours after seeding. $VEGF_{121}$ was added to the cells at concentrations of either 25 or 50 ng/mL. Luciferase expression was measured 24 hours after the addition of $VEGF_{121}$ and 48 hours after the addition of $VEGF_{121}$. The results are shown in Figure 6. Maximum luciferase expression was found in the cells treated with 50 ng/mL of $VEGF_{121}$ at 24 hours post-$VEGF_{121}$ introduction.

IN THE CLAIMS

1. (Amended) A method for determining vascular endothelial growth factor (VEGF) activity in a sample, said method comprising the steps of:

a) contacting a sample to be assayed for VEGF activity with a stable HeLa cell line wherein the stable Hela cell line comprises; ~~comprising cells transfected with~~

1) a reporter vector having an expressible reporter element and a DNA binding site disposed adjacent thereto and,

2) a chimeric ~~transactivatable~~ transactivator vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain which specifically binds to the DNA binding site, and

3) an expression vector encoding a gene for a VEGF receptor ~~detecting the presence of expressed reporter element indicating VEGF activity;~~ and

b) ~~D~~detecting expression of the reporter element, wherein expression of the reporter element indicates VEGF activity.

7. (Amended) A method according to Claim 1, wherein the phosphorylatable protein encoded by the chimeric ~~transactivatable~~ transactivator vector can be phosphorylated by MAPK.

8. (Amended) A method according to Claim 1, wherein the phosphorylatable protein is ~~comprises~~ FLK-1.

9. (Amended) A method according to Claim 1, wherein the gene encoding for the phosphorylatable protein is operably linked to a promoter ~~promoting~~ element.

14. (Amended) A method according to Claim 1, wherein said contacting step further comprises binding VEGF present in the sample with the expressed VEGF receptor.

15. (Amended) A method according to Claim 14, wherein said ~~including~~ contacting the sample step further comprises activating MAPK with the expressed VEGF receptor.

21. (Amended) A method according to Claim 1, wherein the sample comprises cells, tissue, tissue extracts ~~and~~ or combinations thereof.

22. (Amended) A method according to Claim 1, wherein the VEGF activity is detectable at ~~in~~ a VEGF concentration of >1 mg/mL.

23. (Amended) A method according to Claim 1, wherein the VEGF activity is detectable at ~~in~~ a VEGF concentration range between ~~from~~ approximately 1 ng/mL to approximately 200 ng/mL.

24. (Amended) A method according to Claim 1, further comprising the step of incubating the sample with the stable HeLa cell line for a period of time ranging from approximately 4 hours to approximately 24 hours.

25. (Amended) A method according to Claim 1, further comprising the step of incubating the sample with the stable HeLa cell line for a period of time ranging from approximately 10 hours to approximately 20 hours.

29. (Amended) A method for determining whether a candidate compound ~~is useful for modulating~~ modulates VEGF activity, said method comprising the steps of:

(a) providing a cell expressing VEGF;

(b) contacting the VEGF produced by the cell with a candidate compound;

(c) contacting ~~a sample to be assayed for VEGF activity~~ the resulting combination of (a) and (b) with a stable HeLa cell line ~~comprising cells~~ wherein the stable HeLa cell line comprises; ~~transfected with~~

1) a reporter vector having an expressible reporter element and a DNA binding site disposed adjacent thereto; and

2) a chimeric ~~transactivatable~~ transactivator vector comprising a gene encoding a phosphorylatable protein and a DNA binding domain which specifically binds to the DNA binding site; and

3) an expression vector encoding a gene for a VEGF receptor; ~~detecting the presence of expressed reporter element indicating VEGF activity;~~ and

(d) ~~D~~detecting expression of the reporter element, wherein expression of the reporter element indicates VEGF activity;

further wherein altered VEGF activity relative to a cell not contacted with the candidate compound indicates that the candidate compound modulates VEGF activity.

30. (Amended) A method according to Claim 29~~27~~, wherein the reporter vector further comprises a GAL4 binding element.

31. (Amended) A method according to Claim 29~~30~~, wherein the reporter vector comprises a gene encoding for a detectable product.

35. (Amended) A method according to Claim 29, wherein the phosphorylatable protein encoded by the chimeric ~~trans-activatable~~ transactivator vector can be phophorylated by MAPK.

37. (Amended) A method according to Claim 29, wherein the gene encoding for the phosphorylatable protein is operably linked to a promoter ~~promoting~~ element.

42. (Amended) A method according to Claim 29, wherein said ~~including~~ contacting the resulting combination step further comprises activating MAPK with the expressed VEGF receptor.

43. (Amended) A method according to Claim 42, wherein said contacting the resulting combination step further comprises binding VEGF present in the sample with expressed VEGF receptor.

49. (Amended) A method according to Claim 29, wherein the sample comprises cells, tissue, tissue extracts, ~~and~~ or combinations thereof.